REMARKS

Claims 1-11 are pending. New claim 12 is added. Support is found at page 3 lines 10-13, page 10 lines 15-17 and Figure 1. No new matter has been added.

Claims 1-11 stand rejected as obvious under 35 USC § 103 over Moeller et al. (US Patent 6,103,502) in view of Schulz et al. (1997) and Palomares et al. (2000). Applicants respectfully disagree with the rejection for the reasons below.

"Under § 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background the obviousness or nonobviousness of the subject matter is determined." *Graham v. John Deere Co. of Kansas City,* 383 U.S. 1, 17-18, 86 S.Ct. 684, 15 L.Ed.2d 545 (1966).

The Patent Office relies primarily on Moeller et al. as evidence of obviousness. Moeller et al. teaches an ultrafiltration process for purifying a small protein from a cell-free fermentation medium. Moeller states that the prior ultrafiltration processes "are all based on the principle of employing, for filtration of a peptide or a protein, a membrane of molecular weight cut-off ***in or below*** the molecular weight range of the peptide or protein to be retained." Col. 3, lines 4-7, emphasis added. In contrast, Moeller et al. discloses a process in which a protein is concentrated by ultrafiltration using membranes in which the stated molecular weight cut-off of the membrane is ***higher*** than the molecular weight of the protein to be retained by the membrane. Col. 3, lines 36-43.

Moeller et al. provides ***only one*** example of this. Namely, Moeller et al. shows that the small protein hirudin (65 amino acids, molecular weight of 7,000 Dalton) can be retained using ultrafiltration membranes having an exclusion limit of 20,000 Dalton. Col. 1, lines 31-35, col. 4, lines 5-6, and col. 4, line 61 to col. 5, line 47. Furthermore, not only did Moeller et al. show this with only one protein, they also showed this process in only one separation, namely separation from cell culture medium for recombinant production in *Saccharomyces cerevisiae* from which

the cells were removed and the medium was further clarified by 2-stage layer filtration. Col. 4, line 61 to col. 5, line 12. This is hardly a generalized teaching, but rather is a specific showing of one example going against the conventional teaching that filter pores should be smaller than the molecule they are to retain.

Moeller et al. teaches that the cell culture medium is ultrafiltered using this larger-pore membrane and then subjected to diafiltration, i.e. desalinated, until the conductivity κ is ≤ 2.0 mS/cm. Col. 5, lines 26-34. Moeller et al. states that after the diafiltration, the product can be concentrated again. Col. 5, lines 31-33.

Moeller et al. provides no theory or explanation for why hirudin defies theory under these conditions and is retained when such large membrane pore sizes are used.

In contrast, the present claims are directed to methods for concentrating a macromolecule from an aqueous solution comprising the macromolecule and an organic polymer. First the solution is subjected to ultrafiltration to produce a first retentate, and then the conductivity of the first retentate is adjusted such that precipitation of the solution components induced by the organic polymer is substantially prevented or reversed to produce a second retentate solution. Lastly, the second retentate solution is subjected to ultrafiltration to further concentrate the macromolecule.

Applicants addressed the problem of increasing protein concentration in conventional cell-culture based protein manufacturing. During the isolation phase, typically concentration factors of only about 10-20 fold are reached using ultrafiltration because of filterability problems and precipitation when attempts were made to further increase concentration. Specification, page 2, lines 2-6. Applicants discovered surprisingly that a cell culture supernatant comprising the product macromolecules and an organic polymer that co-concentrates with the product macromolecule during ultrafiltration, such as a Pluronic® copolymer, can be greatly concentrated with higher yields than any prior reported process of which Applicants are aware by subjecting the supernatant to an initial ultrafiltration, adjusting the conductivity of the retentate,

and ultrafiltering a second time.

Moeller et al. does not teach ultrafiltration with an organic polymer. Because Moeller et al. does not teach an organic polymer, it clearly does not teach that the polymer is a nonionic block copolymer (claim 6), Pluronic® F-68 (claim 7 in generic terms), or selected from polyoxyethylene-polyoxypropylene-polyoxyethylene triblock copolymers, polyethylene glycol, and antifoam polymers (claim 12).

The Examiner admits that Moeller et al. do not teach adding Pluronic® F-68. She argues, however, that Schulz et al. teach the desirability of ultrafiltration and that Pluronic® F-68 is often a supplement in cell culture media for animal cells to protect the cells from shear stress caused by sparging. She also argues that Palomares et al. teach the effectiveness of Pluronic® F-68 in animal and insect cell lines.

The Examiner reasons that it would have been obvious to the skilled person to use Pluronic® F-68 in the methods taught by Moeller et al. when the method is used to cultivate animal or insect cells. The Examiner finds an expectation of success with using the ultrafiltration/diafiltration/ultrafiltration method of Moeller et al. with Pluronic® F-68 "because Schulz et al. explain that ultrafiltration and diafiltration are one of the most efficient processes recommended as a first step in downstream procedures for recovery of proteins from mammalian cell cultivation and thus use of Pluronic® F-68 is essential in this process and because Palomares et al. teach that said Pluronic® F-68 is also vital for insect cell cultures in an analogous manner to mammalian cell cultures." Office Action, p. 5.

Applicants respectfully disagree with this reasoning. Usually, when a larger cut-off membrane is used than the protein's size, the product loss by passing through the membrane is substantial. There is no explanation for the mechanism by which Moeller et al. avoid this product loss. Thus, the skilled person would assume that the results are specific to hirudin in the yeast expression system using the cell culture medium described and would not be motivated to modify the system at all.

Furthermore, the Patent Office has not cited any reference or provided any reasoning as to why the skilled person would change cell lines from the yeast cells used in Moeller et al. to an insect or mammalian cell. The Patent Office argues that "when cultivating animal and insect cells that the additive Pluronic® F-68 (or similar block nonionic copolymers) *must* be added to the cell cultures" to prevent cell death (Office Action, p. 5). Yet there is no reference or reasoning as to why the skilled person would change from the yeast cell line of Moeller et al. Thus, a prima facie case including motivation to modify has not been made.

Not only would the skilled person reading Moeller et al. not be motivated to change expression systems, but the person also would not be motivated to add Pluronic® F-68. Schulz et al. teaches that Pluronic® F-68 caused additional membrane fouling and thus an increase of process time (abstract and page 4 (Figure 2a showing flux decrease due to membrane fouling)) and that it further complicates downstream processing of pharmaceutical proteins (page 2). Although Palomares et al. teach that Pluronic® F-68 is an effective shear protective agent for animal cells in suspension culture, the teaching of Schulz et al. of the processing problems with ultrafiltration of Pluronic® F-68-containing cell culture medium would direct the skilled person away from modifying Moeller et al. to an animal or insect expression system that would need an agent such as Pluronic® F-68.

Claim 2 of the present invention recites in step 2 "adjusting the conductivity of the first retentate solution such that precipitation of the solution components induced by the organic polymer is substantially prevented or substantially reversed...." The Office Action relies upon the disclosure in Moeller et al. of a diafiltration step in the isolation of hirudin as suggesting this step. Office Action, p. 4. Yet Moeller et al. teach diafiltration for desalination. If salinity is not a problem, then this step could be ignored. For example, the skilled person would be motivated to ignore this diafiltration step if salinity is not a problem for his or her process, such as when subsequent chromatography steps can be performed under the salinity conditions of the retenate. To establish that the invention would have been obvious, the Patent Office must show that not only would the skilled person be motivated to modify Moeller et al. to an insect or mammalian cell expression system and use an organic polymer such as Pluronic® F-68, but also that the

skilled person would then be motivated to continue with a diafiltration step that was only taught for desalination. Such a showing has not been made.

Furthermore, Moeller et al. alone or in combination with the cited references does not render the invention of claims 10 and 11 obvious. Claims 10 and 11 recite the method of claim 1 in which the resulting solution has at least a 50 fold higher (claim 10) or at least a 100 fold higher (claim 11) concentration of the macromolecule than the starting solution. In contrast, Moeller et al. describes an increase in hirudin concentration of only eight fold in total. Col. 5, lines 31-34.

CONCLUSION

For at least the above reasons, Applicants respectfully request withdrawal of the rejections and allowance of the claims.

Filed herewith is a petition for a two-month extension of time with the authorization to charge the requisite fee to the deposit account or credit card of the undersigned. Applicants believe no further fee is due with this response. However, if an additional fee is due, please charge our Deposit Account No. 03-2775, under Order No. 07430-00191 from which the undersigned is authorized to draw.

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Respectfully submitted,

Electronic signature: /Christine M. Hansen/
Christine M. Hansen
Registration No.: 40,634
CONNOLLY BOVE LODGE & HUTZ LLP
1007 North Orange Street
P. O. Box 2207
Wilmington, Delaware 19899-2207
(302) 658-9141
Attorney for Applicant